

# Validation of serum ferritin values by magnetic susceptometry in predicting iron overload in dialysis patients

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## Validation of serum ferritin values by magnetic susceptometry in predicting iron overload in dialysis patients.

**Background.** Guidelines for treating anemia in dialysis patients accept, as high-end range of serum ferritin useful to optimize erythropoietin therapy, values high as 500 to 900  $\mu\text{g/L}$ , on the hypothesis that ferritin might be not representative of iron overload.

**Methods.** A superconducting quantum interference device (SQUID) was used to make direct noninvasive magnetic measurements of nonheme hepatic iron content in 40 dialysis patients treated with intravenous iron, and liver iron content was compared with biochemical markers of iron status.

**Results.** Only 12/40 (30%) patients showed normal hepatic iron content (SQUID  $<400 \mu\text{g/g}$ ), while 32.5% had mild (400 to 1000  $\mu\text{g/g}$ ) and 37.5% severe ( $>1000 \mu\text{g/g}$ ) iron overload, although 28/40 patients (70%) had serum ferritin below 500  $\mu\text{g/L}$ . Among many parameters, hepatic iron content was only correlated with ferritin ( $r = 0.324$ ,  $P = 0.04$ ). The receiver operating characteristic (ROC) analysis showed the best specificity/sensitivity ratio to identify iron overload for ferritin  $>340 \mu\text{g/L}$  ( $W = 0.716$ ). Multivariate logistic regression analysis demonstrated that an increase in serum ferritin of 100  $\mu\text{g/L}$  and female gender were independent variables associated with moderate to severe hepatic iron overload: OR 1.71 (95% CI 1.10 to 2.67) and OR 10.68 (95% CI 1.81 to 63.15), respectively.

**Conclusion.** Hepatic iron overload is frequent in dialysis patients with ferritin below currently proposed high-end ranges, and the diagnostic power of ferritin in indicating true iron stores is better than presumed. Safety concerns should prompt a reevaluation of acceptable iron parameters, focusing on potential gender-specific differences, to avoid potentially harmful iron overload in a majority of dialysis patients, mainly females.

The use of recombinant human erythropoietin (rHuEPO) has made possible the correction of anemia in most uremic patients [1–3]. As rHuEPO-stimulated

erythropoiesis is very demanding, the successful use of rHuEpo to correct anemia requires sufficient iron availability before and during therapy to allow an optimal drug response [4–6]. As virtually all uremic patients on dialysis on rHuEPO receive ongoing maintenance iron supplementation in continuous or intermittent need-based schedules, any associated risk (iron deficiency and iron overload) must be weighted [7].

In the last years, a growing body of studies has evaluated the reliability of “old” and “new” biochemical parameters in interpreting the truth of the iron status: circulating serum ferritin and transferrin saturation (TSAT), the percentage of hypochromic red cells, reticulocyte hemoglobin content, soluble transferrin receptor, and zinc protoporphyrin. However, iron deficiency status seems now easier to be diagnosed than does iron overload status. In fact, not only the “new” markers of iron available for erythropoiesis are early and sensitive tools in diagnosing iron deficiency as perceived at the level of bone marrow progenitors, but also an “old” parameter such as serum ferritin is a more useful gauge of iron status at lower values than at higher ones. At a serum ferritin concentration  $\leq 12 \mu\text{g/L}$ , a status of true iron deficiency may be comfortably forecasted. On the contrary, no alternative biochemical parameters other than serum ferritin have been introduced to look for iron overload, and ultimate validation of this parameter may only be drawn by invasive techniques measuring directly tissue iron content, such as bone marrow or liver biopsy.

In fact, true tissue iron overload may lead to increased serum ferritin really signaling increased iron stores, but other reasons might account for neoplasia, inflammatory or infectious state (serum ferritin being an acute-phase reactant) and tissue damage (for instance liver disease) with cellular lysis and release of tissue ferritin in the blood stream. Inflammatory cytokines may block iron into tissue stores, preventing bone marrow progenitors from using existing wealthy iron stocks, or may increase the rate of secretion of serum ferritin in the blood stream, even in the presence of empty iron stores, so that this circulating

**Key words:** ferritin, transferrin saturation, iron overload, uremia, magnetic susceptometry, gender.

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ferritin will eventually be not representative of stored iron. In both cases, laboratory parameters might show a dissociation between increased serum ferritin concentration and signaling of iron insufficiency from bone marrow progenitors. As uremia is regarded as an inflammatory status, it has been suggested that high serum ferritin concentrations in uremic patients do not represent high iron stores available for erythropoiesis, but belong to the above-mentioned categories [8–11].

Most guidelines for treating anemia in uremic patients on rHuEPO agree on the low-end range of serum ferritin at which rHuEPO has to be started, but disagree on high-end range, which has not to be exceeded to avoid risk of iron toxicity. Values as high as 800 and 1000  $\mu\text{g/L}$  had been accepted [12], but lower values (500  $\mu\text{g/L}$ ) have been recommended [13, 14].

Lights to clarify this important issue may only come from measurements of tissue iron content, but invasive techniques cannot be proposed for clinical sequential studies.

We measured the liver iron concentration by in vivo determination of hepatic magnetic susceptibility by SQUID (superconducting quantum interference device) biosusceptometer. The SQUID is predominantly determined by the magnetic volume susceptibility of the paramagnetic ferritin/hemosiderin iron in the liver. It has been validated in comparison to percutaneous liver biopsy and is the only noninvasive method yielding results that are quantitatively equivalent to and that can be used interchangeably with those obtained by chemical analysis of biopsy tissue [15–23].

The aim of our work was to verify hepatic iron stores in uremic patients on dialysis as measured by the SQUID and to validate the reliability of biochemical parameters markers of iron status, thanks to their correspondence with the hepatic nonheme iron content measured by the SQUID.

## METHODS

As SQUID is very expensive, a maximum of 50 evaluations was allowed for this research. Out of 40 patients on chronic hemodialysis from at least 6 months at our Self-Care Dialysis Center, 30 were eligible for this study and agreed to participate. Another 10 patients were recruited from the 60 patients on hemodialysis since at least 6 months at our Hospital Dialysis Center. The last 10 allowed evaluations by SQUID were reserved in order to have the possibility of performing a sequential control over time in 10 cases.

Exclusion criteria were presence of overt inflammatory and infectious disease, recent major bleeding, hospitalization, surgery, poor compliance, recent transfusions, malignancies, steel plates, artificial joints, and cardiac pacemakers (because of contamination of the magnetic

field employed by the SQUID). Informed consent was obtained from all the patients.

In agreement with the European and Italian Guidelines for anemia in hemodialysis patients, current treatment schedules in our center were the following: *target*, hemoglobin 10 to 11 g/dL; TSAT 20% to 50%, and serum ferritin 200 to 500  $\mu\text{g/L}$ , respectively; *monitoring*, hematocrit testing every week and hemoglobin every 3 months, iron testing every 3 months, sampled after 1 week after a dose; *iron therapy*, continuous supplementation by intravenous sodium ferric gluconate complex 31.25 mg (“Ferlixit” 62.5 mg/amp) (Nattermann & Cie GmbH, Colonia, Germany) dissolved in saline 20 mL infused in 15 minutes at the end of hemodialysis once or twice a week.

All 40 patients who entered the study (15 females and 25 males) had been treated with iron therapy. Ten were on maintenance intravenous iron from at least 6 months at the moment of the evaluation by SQUID, and 30 had suspended iron therapy from at least 2 months following 15 months of continuous supplementation, after last iron tests showing serum ferritin value  $>500 \mu\text{g/L}$ . Dialysis adequacy was serially measured by Kt/V and resulted  $>1.2$  in all patients. Nobody tested positive for hepatitis B surface antigen, while 14 patients tested positive for anti-hepatitis C virus antibodies. Liver enzymes were persistently normal in all the 40 patients of the study. Thirteen patients underwent vitamin C therapy (500 mg intravenous at the end of dialysis once a week) owing to serum ascorbate value below the normal range ( $<2.5 \text{ mg/mL}$ ); rHuEPO was administered in 34 out of 40 patients as subcutaneous injection at the end of the hemodialysis session in doses ranging from 1000 to 12,000 U/week.

## Study design

*Study 1. Cross-sectional study.* All 40 patients underwent the evaluation by SQUID without changing the ongoing therapeutic schedules. Patients on continuous iron replacement received iron dose 1 week before the SQUID examination. All patients underwent blood sampling for biochemical examination in the same day of the SQUID analysis.

Following previous validation of measurements of hepatic nonheme iron content by the SQUID, the patients were grouped in (1) normal hepatic iron content ( $<400 \mu\text{g/g}$  wet weight); (2) mild iron overload ( $>400 < 1000 \mu\text{g/g}$  wet weight); (3) moderate iron overload ( $>1000 < 2000 \mu\text{g/g}$  wet weight); and (4) severe iron overload ( $>2000 \mu\text{g/g}$  wet weight). This grouping was based on Torino SQUID activity (Tristan Technologies, Inc., San Diego, CA, USA). Ninety-one normal adults (mean age  $46.2 \pm 13.9$  years, range 21.2 to 72.2 years) with normal iron parameters and negative for the main genetic hemochromatosis mutations (C282Y and H63D) had a mean hepatic iron content of  $223 \pm 125 \mu\text{g/g}$  wet weight

(range 0 to 394  $\mu\text{g/g}$  wet weight); 2011 adult patients with various degree of iron overload from different pathologic conditions (thalassemias, hemoglobinopathies, genetic hemochromatosis, liver diseases, chronic anemias, malignancies) ranged from 407 to 9856  $\mu\text{g/g}$  wet weight. The limits for mild, moderate, and severe iron overload have been chosen on our experience, in accordance with the literature [16, 48].

For calculations, hepatic iron content  $>400$   $\mu\text{g/g}$  wet weight was considered "excess iron," and biochemical indirect iron parameters were evaluated for their diagnostic power in recognizing excess iron.

**Study 2. Longitudinal study.** The 10 patients with the highest iron content as evaluated by SQUID were chosen to repeat a second SQUID examination 6 months later after iron supplementation withdrawal.

Measurement of nonheme hepatic iron concentration by SQUID was accomplished as follows. Liver iron concentrations were derived from the specific ferritin iron susceptibility. The Torino SQUID biosusceptometer (Model 5700; Tristan Technologies, Inc.) is a three-channel system. The "next generation" biosusceptometer planned for Oakland and Torino has been designed by Dr. R.F. (Hamburg), in collaboration with Dr. D.P. and Dr. R.F. of Tristan Technologies, Inc. It differs in liquid elium container (dewar) structure, coil design, water bag size, and weight and software design from the Cleveland and Hamburg instruments. Specificity should be improved by inclusion of an additional surface-sensitive detector coil and sensitivity should be improved by developing a smaller dewar gap distance using an adjustable tail design. Dr. R.F., working in collaboration with Dr. A.P. and F.L. (Torino), Dr. D.P. and Dr. R.F. (Tristan Technologies, Inc.), and the Thalassemia Consortium are critically evaluating the engineering advances incorporated into the "next generation" biosusceptometer utilizing the large group of thalassemia and controls patients receiving simultaneous liver biopsies. The correlation between the liver iron biopsy and data obtained from the spectroscopy measurement was highly significant ( $r = 0.982$ ,  $P < 0.00005$ , personal communication).

Effective magnetic biosusceptibility has been measured by a protocol developed and validated by Dr. R.F. Those measurements involve (1) ultrasound assessment of liver volume, optimal site for measurement, and skin to liver surface distance; (2) susceptibility measurement; and (3) computer analysis of data. The mean iron concentration value was calculated from all selected runs in both independent detector channels. SQUID procedures were performed as described elsewhere [24–27].

### Biochemical parameters

Serum ferritin (normal value in our laboratory 25 to 340  $\mu\text{g/L}$  for males and 15 to 150  $\mu\text{g/L}$  for females) was

evaluated by immunofluorometric assay, iron and transferrin concentration by iron guanidine hydrochloride/ferrozine assay, and by nephelometric assay, respectively. TSAT was calculated by the formula:  $[\text{iron } (\mu\text{g/dL}) / [\text{transferrin } (\text{mg/dL}) \times 1.4]$ . Hematologic parameters, including hypochromic erythrocytes and reticulocyte hemoglobin content were measured by hematology analyzer (ADVIA 120; Bayer Diagnostics, Colonia, Germany). Erythrocytes with hemoglobin concentration  $<28$   $\text{g/dL}$  were considered to be hypochromic and values were expressed as percentage of total red blood cells. The reticulocyte hemoglobin content was calculated by the product of hemoglobin concentration and reticulocyte volume and a value  $<32$   $\text{pg/mL}$  was considered abnormal, based on the mean of the normal population. Soluble transferrin receptors (sTfR) were measured by commercially available enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Inc., Minneapolis, MN, USA) (normal range 8.7 to 28.2  $\text{mmol/L}$ ). C-reactive protein (CRP) was measured by the nephelometric method (Dade Boehringer, Mannheim, Germany) (normal value  $<3$   $\text{mg/L}$ ). Intra- and interassay variation coefficients for all iron status laboratory indices were  $<5\%$ . Iron parameters considered in the work were measured in a nonsteady-state system, as the patients were actively using iron to produce red cells. For the calculation of the total body iron stores the following formula has been used:  $[\text{serum ferritin } (\mu\text{g/L}) \times 120 \times \text{kg/body weight}]$  in agreement with previous reports suggesting that, although 1  $\mu\text{g/L}$  plasma ferritin in the normal adult is equal to 8 to 10  $\text{mg}$  tissue iron stores, when comparing individuals of differing body weight, the conversion to 120  $\mu\text{g/kg}$  storage iron is preferable [28].

**HFE gene mutations: DNA study.** The C282Y and H63D mutations of HFE gene were investigated by two specific and standardized kits, produced by the Vienna Lab, and purchased by Nuclear Laser Medicine SRL Settala (MI) Italy, as previously described [29].

For the calculations in this study, C282Y heterozygous, H63D heterozygous, and H63D homozygous were all considered as "HFE gene mutation."

### Statistics

All data are expressed as mean  $\pm$  standard deviation unless otherwise detailed. Differences between groups were evaluated by nonparametric Mann-Whitney  $U$  test, analysis of variance (ANOVA) test, and Bonferroni test for the analysis of discrete and continuous variables, respectively. To identify optimal test and threshold values for predicting hepatic iron excess, receiver operating characteristics (ROC) curve analysis was performed by computing sensitivity and specificity of the different tests at various cut-off levels. Sensitivity was defined as the percentage of patients with hepatic iron excess having a positive biochemical test (true positive) (TP) and specificity

**Table 1.** Main clinical, biochemical, and tissue parameters of the patients divided according to hepatic nonheme iron concentrations as measured by superconducting quantum-interference device (SQUID)

	Nonheme iron stores				P value
	Overall (N = 40)	Normal (N = 12)	Mild overload (N = 13)	Moderate overload <sup>a</sup> (N = 15)	
Age years	53 ± 14 (30–79)	53 ± 15 (31–79)	57 ± 14 (32–79)	51 ± 12 (30–70)	0.59
Gender males/females	25/15	11/1	9/4	5/10	0.007
Dialysis age months	116 ± 117 (10–402)	88 ± 96 (10–359)	74 ± 79 (19–276)	174 ± 140 (18–402)	0.04
Iron dose mg/kg <sup>b</sup>	11 ± 9 (4–47)	14 ± 13 (5–47)	10 ± 8 (4–35)	9 ± 4 (4–19)	0.37
EPO therapy % patients	85	75	92	87	0.47
EPO dose U/week <sup>b</sup>	4765 ± 2965 (1000–12,000)	4667 ± 3162 (2000–12,000)	5417 ± 3528 (1000–12,000)	4231 ± 2315 (2000–8000)	0.62
Anti-hepatitis C virus positive%	35	42	15	47	0.19
Vitamin C therapy %	33	17	46	33	0.29
C-reactive protein mg/L	6.2 ± 8.3 (1–49)	5 ± 4.7 (1–15)	9.3 ± 13.4 (2–49)	4.5 ± 3.1 (2–13)	0.28
Serum albumin g/dL	3.6 ± 0.3 (3–4.2)	3.5 ± 0.2 (3.1–3.7)	3.7 ± 0.4 (3–4.2)	3.6 ± 0.3 (3.1–4.2)	0.25
Hemoglobin g/dL	11.4 ± 1.7 (7–15.1)	11.8 ± 1.8 (10–15.1)	10.3 ± 1.8 (7–13.1)	12 ± 1.1 (10–14)	0.016
Ferritin µg/L	361 ± 224 (14–886)	245 ± 183 (14–561)	329 ± 174 (51–609)	482 ± 246 (101–886)	0.016
Serum iron µg/dL	73 ± 34 (22–190)	81 ± 47 (22–190)	65 ± 33 (28–126)	74 ± 21 (32–112)	0.49
Transferrin mg/dL	163 ± 33 (106–266)	176 ± 46 (124–266)	157 ± 28 (106–227)	157 ± 20 (128–194)	0.22
TSAT %	33 ± 15 (7–86)	34 ± 20 (7–86)	31 ± 15 (9–58)	34 ± 8 (17–44)	0.78
Hypo %	3.8 ± 8.3 (0–43)	8.3 ± 13.9 (0–43)	2.1 ± 3.1 (0.2–11)	1.7 ± 2.7 (0.1–11)	0.07
CHr pg	32.3 ± 2.9 (22.3–37.1)	31.5 ± 4.5 (22.3–37.1)	32.9 ± 2.3 (29.9–37)	32.3 ± 1.9 (27.8–34.6)	0.48
Soluble transferrin receptors nmol/L	23.4 ± 13.1 (9.4–85.9)	28.9 ± 20.9 (9.9–85.9)	20.7 ± 5 (14.3–30)	21.5 ± 8.7 (9.4–42.4)	0.23
Soluble transferrin receptors/log ferritin	4.8 ± 5.1 (1.5–32.5)	7.4 ± 8.7 (1.6–32.5)	3.8 ± 1.4 (2.2–7.6)	3.6 ± 1.5 (1.5–6.7)	0.11
HFE gene mutation % patients <sup>c</sup>	25	42	23	13	0.24
SQUID µg/g wet weight	832 ± 653 (0–3198)	230 ± 130 (0–373)	630 ± 189 (402–951)	1488 ± 579 (1034–3198)	0.02
Iron stores g <sup>d</sup>	2.7 ± 1.6 (0.1–5.8)	1.9 ± 1.3 (0.1–3.5)	2.4 ± 1.2 (0.4–4.2)	3.5 ± 1.9 (0.8–5.8)	0.02

Abbreviations are: EPO, erythropoietin; TSAT, serum transferrin saturation; Hypo, hypochromic red cells; CHr, hemoglobin reticulocyte content.

Normal value <400 µg/g wet weight; mild overload 400 to 1000 µg/g wet weight; moderate overload >1000 µg/g wet weight.

<sup>a</sup>Included one patient with severe iron overload (>2000 µg/g wet weight); <sup>b</sup>Mean value over the last 6 months; <sup>c</sup>Patients C282Y heterozygous, H63D heterozygous, and H63D homozygous were all considered as "HFE gene mutation"; <sup>d</sup>Calculated by the formula: serum ferritin × 120 µg × body weight (kg).

as the percentage of patients with hepatic normal iron having a negative biochemical test (TN). Tests showed discriminative ability when the area under the curve (W) was significantly different from 0.5.

Odds ratio (OR) and 95% CI were estimated by univariate and multivariate logistic regression model. A *P* value < 0.05 was considered as statistically significant.

## RESULTS

### Baseline demographic characteristics of the studied population

Seventy percent of the patients (28/40) had serum ferritin values below the high-end normal range of the European and Italian Guidelines (<500 µg/L), and only two patients (5%) had levels higher than 650 µg/L (Table 1).

Nevertheless, only 12 (30%) patients had hepatic iron stores within the normal range (<400 µg/g wet weight), while a mild-to-severe hepatic iron overload was measured in the other 28 (70%): mild in 13 (32.5%), moderate in 14 (35%), and severe in 1 patient (2.5%).

The group with moderate-to-severe iron overload was not significantly different for frequency of subjects still undergoing iron supplementation, total iron amount infused in the last 6 months, anti-hepatitis C virus positivity, vitamin C therapy, CRP, serum albumin concen-

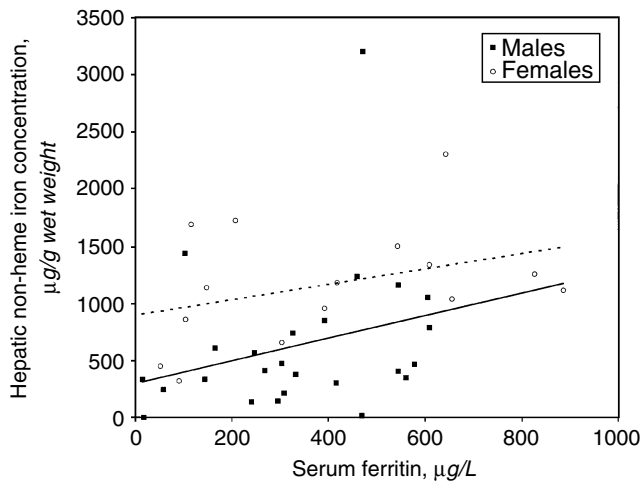
trations, and HFE gene mutations (Table 1). Conversely, there was a significantly increased number of females, longer duration of dialysis, and higher serum ferritin level. Hemoglobin value was significantly lower in the group with mild overload, but was similar in the two groups with normal and highest iron content (Table 1).

The number of patients with TSAT <15% was 3/21 (14%) in the subgroup with serum ferritin <340 µg/mL and 0/19 in the subgroup with serum ferritin >340 µg/mL. Furthermore, in the subgroup of patients with SQUID values below and above the high end of the normal range (400 µg/g wet weight), the number of patients with TSAT lower than 15% was 1/12 (8%) and 2/28 (7%), respectively.

### Correlation between biochemical parameters and SQUID and within biochemical parameters

Among the biochemical parameters, only serum ferritin showed a weak significant correlation with the hepatic nonheme iron concentrations as measured by the SQUID (*r* = 0.325, *P* = 0.041). The amount of variance explained by serum ferritin is about 10.5% (Fig. 1).

Negative correlations were found between ferritin and TSAT, on one side, and all the makers of iron deficiency at the level of bone marrow progenitors on the other side (Table 2).



**Fig. 1. Correlation between serum ferritin concentration and hepatic nonheme iron content as measured by the superconducting quantum interference device (SQUID) ( $r = 0.325$ ,  $P = 0.041$ ).**

### ROC curve

Diagnostic power of serum ferritin and TSAT in signalling hepatic excess iron was calculated by a ROC curve (Fig. 2). Serum ferritin ( $W = 0.761$ ), unlike TSAT ( $W = 0.446$ ), showed discriminative ability. Serum ferritin demonstrated a marginal utility, as none of the cut-off values had a high level of utility (both sensitivity and specificity  $>80\%$ ). The best threshold value to detect hepatic excess iron was  $>340 \mu\text{g/L}$ , associated with 75% specificity and 57% sensitivity. When the cut-off level was increased to a value of  $>450$ ,  $>500$ , or  $>600 \mu\text{g/L}$ , the specificity becomes 82%, 92%, and 100%, respectively.

### Logistic regression analysis

Univariate logistic regression analysis showed that serum ferritin, female gender, and dialysis vintage were the only factors associated with mild and moderate to severe hepatic iron overload (Table 3). A multivariate logistic regression model, including gender and ferritin, confirmed that serum ferritin  $>500 \mu\text{g/L}$  and female gender were independent risk factors for moderate-to-severe hepatic iron overload: OR 10.75 (95% CI 1.54 to 75.16) and OR 10.47 (95% CI 1.81 to 60.73), respectively. Although not reaching the level of statistical significance, serum ferritin  $>340 \mu\text{g/L}$  was also an independent risk factor for having moderate to severe iron overload: OR 5.27 (95% CI 0.57 to 48).

Additional studies showed that, in multivariate analysis adjusted by sex, an increase of  $100 \mu\text{g/L}$  of serum ferritin was an independent risk factor for having mild (OR 1.68, 95% CI 1.03 to 2.75) and moderate-to-severe (OR 1.71, 95% CI 1.10 to 2.67) hepatic iron overload. Female gender remained the highest risk factor: OR 15.31 (95%

CI 1.41 to 165) and OR 10.68 (95% CI 1.81 to 63.15), respectively.

### Study 2. Longitudinal study

In the 10 patients who were reevaluated by SQUID after 6 months without iron supplementation, there was a significant decrease in hepatic iron concentrations ( $1302 \pm 746 \mu\text{g/g}$  wet weight to  $654 \pm 272 \mu\text{g/g}$  wet weight,  $P < 0.01$ ), as well as in serum ferritin levels ( $550 \pm 197 \mu\text{g/L}$  to  $442 \pm 155 \mu\text{g/L}$ ,  $P < 0.05$ ).

### DISCUSSION

Our study correlates for the first time in dialysis patients the indirect parameters that estimate the iron status with a direct measurement of liver iron obtained by the SQUID.

When we planned this study, we did not forecast these results. In fact, in agreement with the guidelines, our policy in iron supplementation was not aggressive, and most of our patients were expected to have normal hepatic iron concentration. Surprisingly, in 70% of subjects who were on low-dosage continuous intravenous iron supplementation (or had even stopped iron supplementation from at least 2 months) and have moderately high serum ferritin, there was evidence of excess iron in liver storage as demonstrated by SQUID.

Notwithstanding theoretical doubts on reliability of serum ferritin, our study first demonstrates that high serum ferritin concentration is an independent predictor for having overloaded hepatic iron stores in dialysis patients, in agreement with that reported in children with transfusion siderosis [18, 30]. Furthermore, the risk of having iron overload is increased already at serum ferritin values  $>340 \mu\text{g/L}$  (the high-end normal range for men in our laboratories). In fact, the poor efficiency of high values of serum ferritin in the ROC curve was due to the low sensitivity, but specificity was high. At a cut-off level of  $340 \mu\text{g/L}$ , poor efficiency means that also some patients with serum ferritin  $<340 \mu\text{g/L}$  have hepatic excess iron, coupled with a majority of patients with serum ferritin  $>340 \mu\text{g/L}$ . Therefore, even  $340 \mu\text{g/L}$  as high end range might be too high in order to completely exclude iron overload.

The fact that hepatic iron was not related to the iron amount received over the last 6 months might be explained by the different handling of supplemented iron in different cases, due to different erythropoietic demand and different loss. As it has been previously underscored, iron parameters considered in this work were measured in a nonsteady-state system. Our study does not confirm that, at least in uremic patients on dialysis in good clinical conditions and without clinically overt inflammation, moderately high serum ferritin is “not representative”

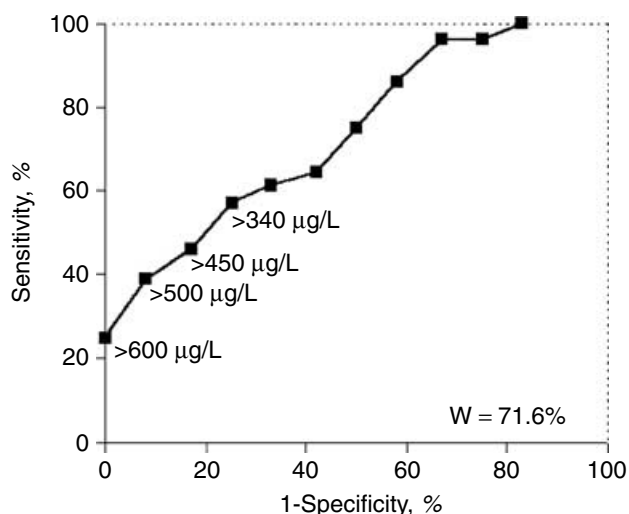
**Table 2.** Pearson correlation coefficients among all iron status markers considered in this study

	SQUID	Ferritin	Iron	Transferrin	TSAT	Hypo	CHr	Soluble transferrin receptor	Soluble transferrin receptor/log ferritin
Ferritin	<i>r</i> 0.325 <b><i>P</i> 0.041</b>								
Iron	<i>r</i> -0.051 <i>P</i> 0.75	<i>r</i> 0.343 <b><i>P</i> 0.03</b>							
Transferrin	<i>r</i> -0.179 <i>P</i> 0.27	<i>r</i> -0.295 <i>P</i> 0.06	<i>r</i> 0.137 <i>P</i> 0.4						
TSAT	<i>r</i> -0.017 <i>P</i> 0.92	<i>r</i> 0.421 <b><i>P</i> 0.007</b>	-	-					
Hypo	<i>r</i> -0.189 <i>P</i> 0.24	<i>r</i> -0.401 <b><i>P</i> 0.01</b>	<i>r</i> -0.363 <b><i>P</i> 0.021</b>	<i>r</i> 0.163 <i>P</i> 0.32	<i>r</i> -0.388 <b><i>P</i> 0.013</b>				
CHr	<i>r</i> -0.069 <i>P</i> 0.67	<i>r</i> 0.320 <b><i>P</i> 0.044</b>	<i>r</i> 0.508 <i>P</i> 0.001	<i>r</i> -0.128 <i>P</i> 0.43	<i>r</i> 0.534 <b><i>P</i> &lt; 0.001</b>	<i>r</i> -0.846 <b><i>P</i> &lt; 0.001</b>			
Soluble transferrin receptors	<i>r</i> -0.100 <i>P</i> 0.54	<i>r</i> -0.385 <b><i>P</i> 0.014</b>	<i>r</i> -0.442 <b><i>P</i> 0.004</b>	<i>r</i> 0.392 <b><i>P</i> 0.012</b>	<i>r</i> -0.529 <b><i>P</i> &lt; 0.001</b>	<i>r</i> 0.661 <b><i>P</i> &lt; 0.001</b>	<i>r</i> -0.667 <b><i>P</i> &lt; 0.001</b>		
Soluble transferrin receptors/log ferritin	<i>r</i> -0.187 <i>P</i> 0.25	-	<i>r</i> -0.376 <b><i>P</i> 0.017</b>	<i>r</i> 0.459 <b><i>P</i> 0.002</b>	<i>r</i> -0.475 <b><i>P</i> 0.002</b>	<i>r</i> 0.658 <b><i>P</i> &lt; 0.001</b>	<i>r</i> -0.608 <b><i>P</i> &lt; 0.001</b>	-	
Iron stores <sup>a</sup>	<i>r</i> 0.366 <i>P</i> 0.02	-	<i>r</i> 0.253 <i>P</i> 0.12	<i>r</i> -0.318 <b><i>P</i> 0.046</b>	<i>r</i> 0.348 <b><i>P</i> 0.028</b>	<i>r</i> -0.389 <b><i>P</i> 0.013</b>	<i>r</i> 0.248 <i>P</i> 0.12	<i>r</i> -0.288 <i>P</i> 0.07	<i>r</i> -0.415 <b><i>P</i> 0.008</b>

Abbreviations are: SQUID, superconducting quantum interference device; TSAT, serum transferrin saturation.

Statistically significant *P* values are in bold (*P* < 0.05).

<sup>a</sup>Calculated by the formula: serum ferritin × 120 μg × body weight (kg).



**Fig. 2. Receiver operating characteristics (ROC) curve analysis performed by computing sensitivity and specificity of serum ferritin for predicting hepatic iron excess at various cut-off levels.** Serum ferritin showed discriminative ability. The best threshold value for serum ferritin to detect hepatic excess iron was >340 μg/L, associated with 75% specificity and 57% sensitivity. The area under the curve (W) is significantly different from 0.5.

of iron overload, and therefore, that “moderately high serum ferritin concentrations may not be a reliable indication for withholding iron administration in uremic patients” [31]. We cannot exclude that uremic inflammation may had enhanced hepatic iron uptake and, perhaps, reduced iron release from hepatic stores, but excess iron is present in hepatic stores, when serum ferritin is moder-

**Table 3.** Univariate logistic regression analysis evaluating the odds ratios of various clinical and biochemical factors for having two degrees (mild and moderate) of increased hepatic iron content

Clinical and biochemical factors	Mild hepatic non-heme iron overload (>400 < 1000 μg/g wet weight) OR (95% CI)	Moderate to severe hepatic nonheme iron overload (>1000 μg/g wet weight) OR (95% CI)
Gender (female)	11 (1.25–97)	8 (1.87–34.22)
Body weight kg	0.98 (0.92–1.05)	0.97 (0.91–1.04)
Dialysis vintage years (>3 < 10 > 10)	(0.20–5.87)	(0.48–45.55)
Iron therapy mg/kg (>7 < 10 > 10)	1.50 (0.22–10.08) (0.41–11.30)	11.20 (1.04–120.36) (0.27–5.25)
Serum ferritin μg/L (>340 < 500 > 500)	0.65 (0.11–3.41) (0.29–11.87)	1.60 (0.29–8.73) (0.50–20.3)
HFE gene mutation	8.25 (0.89–76.12)	8.50 (1.68–42.98)
Hepatitis C virus seropositive	0.30 (0.06–1.36)	0.32 (0.05–1.80)
Vitamin C therapy	0.67 (0.16–2.67)	2.25 (0.59–8.57)
C-reactive protein (>3 mg/dL)	3.23 (0.59–17)	1.06 (0.27–4.15)
Serum albumin (<3.5 g/dL)	0.91 (0.23–3.58)	1.56 (0.42–5.72)
	0.4 (0.12–1.83)	0.72 (0.19–2.64)

ately high. However, our criteria inclusions for patients excluded many types of chronic hemodialysis patients (including “hospitalization, recent surgery and transfusions, infectious diseases, malignancies”). These patients would likely have higher ferritin, and therefore, further studies are needed to clarify if our results may be applied also in these cases.

As to the clinical meaning of hepatic iron overload, this topic was beyond the scope of our work. However, many studies pointed to the fact that hepatic iron overload may predict more diffuse iron overload in other organ and tissues [32], and focused on toxicity for immune system [33–36], liver function [37–40], and cardiovascular system [41, 42], not only in thalassemia and genetic hemochromatosis, but also in uremia. SQUID is able to quantify accurately intracellular ferritin iron in organs, without differentiation between reticuloendothelial system and parenchymal cells. On the other side, it is also true that in the liver the reticuloendothelial system is well represented.

Another important point to be drawn from our results is the crucial role of female gender in increasing the risk of iron overload. The phrase “clinical guidelines ignore gender differences” [43] strictly applies to guidelines for optimizing iron therapy in treating anemia of uremic patients. The appropriate ferritin cut-off value to use for defining iron deficiency in males and females is under debate [44–47], and guidelines should not forget gender-specific cut-off points to evaluate iron overload in uremic people treated with rHuEPO, as the risk for having iron storage overload is more than 10 times increased in women in our study.

## CONCLUSION

Thanks to a noninvasive procedure, just needing a low field magnet to be placed over the liver, we may know the true iron status for that concerns hepatic tissue stores in dialysis patients chronically supplemented with maintenance intravenous iron. We realize that, due to the cost and lack of availability, SQUID will not be a useful tool clinically for many patients. However, as “biomagnetic susceptometry provides the only noninvasive method to measure tissue iron stores that has been calibrated, validated, and used in clinical study” [23], we planned to have use of the unique possibility offered from the SQUID in our city in order to explore the vexing area of the reliability of indirect parameters in indicating iron stores.

While all the other parameters of iron status (including soluble transferrin receptors and hypochromic red cells) lack any correlation, serum ferritin is validated as roughly representative of hepatic iron store at least in hemodialysis patients without overt inflammation. In these cases, serum ferritin can be clinically used for the monitoring of iron stores, and when its value exceeds 500 µg/L the risk for having moderate to severe storage iron overload is 10 times increased (and further 10 times more in females) independently from HFE gene mutations, CRP values, and hepatitis C virus seropositivity.

Optimal correction of anemia would be facilitated by more specific gender-tailored guidelines, but advocating an aggressive approach to iron replacement in the hope of

improving anemia and maximize rHuEPO efficacy casts some doubts on the potential intermediate and long-term risks. Our data suggest that the high end ranges of serum ferritin proposed from clinical guidelines for optimizing iron therapy in uremic patients treated with rHuEPO should be lowered, perhaps to those of the general population, at least in stable patients in good clinical conditions. We feel that the goal of iron therapy is to optimize rHuEPO efficacy and not the iron status is debatable, and claims that clinicians do not need to be too alarmed by high serum ferritin levels provoke worry.

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